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Compound-specific isotope analysis (CSIA) of micropollutants in the environment — current developments and future challenges

Martin Elsner[1] and Gwenaël Imfeld[2]

Over the last decade, the occurrence of micropollutants in the environment has become a worldwide issue of increasing concern. Compound-specific stable-isotope analysis (CSIA) of natural isotopic abundance may greatly enhance the evaluation of sources and transformation processes of micropollutants, such as pesticides, personal care products or pharmaceuticals. We summarize recent advances from laboratory studies, review current limitations and analytical challenges associated with low concentrations and high polarity of micropollutants, and delineate the potential of micropolluant CSIA for field applications. We highlight future challenges and prospects regarding source apportionment, identification of biotic and abiotic transformation reactions on a mechanistic level, as well as integrative evaluation of degradation hot spots on the catchment scale. Such advances may feed into a framework for risk assessment of micropollutants that includes CSIA.

Addresses
[1] Institute of Groundwater Ecology, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
[2] Laboratoire d'Hydrologie et de Géochimie de Strasbourg (LHyGeS), UMR 7517, Université de Strasbourg/EOST, CNRS, 1 rue Blessig, 67084 Strasbourg Cedex, France

Corresponding author: Elsner, Martin (martin.elsner@helmholtz-muenchen.de)

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Introduction

Today, ground and surface water resources are impacted by numerous organic chemicals. Besides legacy contaminations by petroleum and chlorinated hydrocarbons, polar organic chemicals, typically detected at low concentrations (ng/L to μg/L), are receiving increasing attention (Table 1) [1]. These 'emerging contaminants' [2,3] or 'micropollutants' [1], such as pesticides, pharmaceuticals and consumer care products, are used ubiquitously and enter the environment through agricultural inputs or discharges of treated effluent from waste water treatment plants (WWTPs).

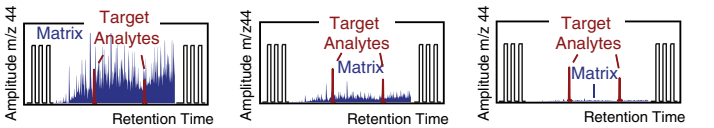
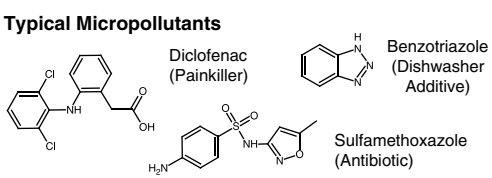
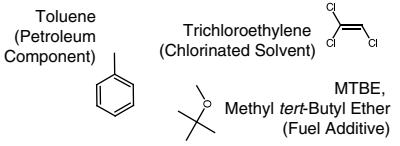
If water scarcity intensifies and water reuse increases [4], and if in addition micropollutants are incompletely removed in engineered water treatment systems, they may accumulate in environmental compartments such as surface waters, sediments and aquifers [2,3,5,6,7]. While research on micropollutants focuses on toxicity, degradability, degradation pathways and transformation products, novel management strategies that rely on their natural and engineered degradation [8,9] and follow the precautionary principle are warranted. Even though micropollutants such as pesticides undergo degradation tests prior to use, the recurrent detection of herbicides in groundwater emphasizes the difficulties to extrapolate laboratory tests to environmental conditions ([10••] and refs. cited therein).

A large body of information on micropollutants degradation is available from laboratory tests and lysimeter studies, and significant advances have been made to identify micropollutant degradation under environmental conditions [2,10••,11], screen their transformation products [7,12] to determine transformation pathways [13,14,15], and predict their transformation rates [5,16]. However and beyond the outcome of regulatory testing, current approaches are limited in their ability to, firstly, predict over relevant long time scales the degradation of micropollutants in the environment. A second limitation is the evaluation, beyond environmental monitoring, of the prevailing dissipation zones and periods ('hot/cold-spots and moments') in integrative studies on the catchment scale. Finally, the drivers controlling the performance and reliability of micropollutant removal in engineered treatment systems (drinking water production, tertiary WWTPs) warrant better characterization.

Research on legacy compounds at contaminated sites has shown that compound-specific isotope analysis (CSIA) may provide a complementary line of evidence to tackle pollutant degradation in environmental systems at different temporal and spatial scales [17,18,19,20]. Gas or liquid chromatographs (GC, LC) coupled to an online conversion reactor and, subsequently, to an isotope ratio mass spectrometer (IRMS) enable precise measurement of naturally occurring stable isotopes (such as $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, $^{37}Cl/^{35}Cl$, $^{81}Br/^{79}Br$) within organic molecules [21]. Isotope ratios (expressed here for carbon) are typically denoted as $\delta^{13}C$ values relative to an international reference material:

Table 1

Comparison of micropollutants with legacy contaminants and consequences for compound-specific isotope analysis.

	Micropollutants / Emerging Contaminants	Legacy Contaminants	Consequences for Compound-specific Isotope Analysis of Micropollutants
	Pharmaceuticals / Consumer Care Products  Pesticides	Petroleum Hydrocarbons / Chlorinated Hydrocarbons 	
Occurrence in the Environment			
Contamination Source	Typically non-point source	Typically point source	- Multiple sources in time and space require careful sampling schemes for source identification - Use of mixing models for multiple sources and sinks
Typical Concentrations	pMol/L — ng/L — nMol/L — µg/L — µMol/L — mg/L		
Typical Amounts of Water To Be Extracted for Compound-specific Isotope Analysis (CSIA)	10 L- 100 L — 1 L-10 L — 100 mL-1 L — 10 mL-100 mL		- Time consuming extraction and sample clean-up due to large volumes of water sample and matrix interferences
Matrix Interferences: Typical Gas Chromatograms of Carbon CSIA without prior Clean-up			
Chemical Properties			
Polarity			- Gas chromatographic performance and online conversion critical for GC-IRMS of micropollutants, derivatization or LC-IRMS may be needed
Volatility			- Enrichment by solid phase extraction possible
Molecular Size			- C isotope fractionation of larger molecules (compound average) becomes small compared to isotope effect in reacting bond
Elements Present in Chemical Structures	Multiple heteroatoms (N, Cl, S, Br, O...)	Mostly restricted to C, H, Cl	- Opportunity for multi-element CSIA with potential to distinguish sources and transformation pathways
Characteristics of Biodegradation			
Knowledge about Degradation Pathways	Often unknown	Often well-established	- Lack of mechanistic scenarios to explain the nature of observed isotope effects
Microbial Metabolism	Potentially co-metabolic: non-selective transformation of substrates below induction thresholds of catabolic pathways	Metabolic: selective substrate use, induction of specific catabolic pathways	- Versatility and concentration-dependence of degradation pathways to be considered for interpretations
Mass Transfer and Bioavailability LImitations	More relevant in the µg/L to ng/L range	Less relevant in the mg/L to µg/L range	- Difficulty of retrieving isotopic enrichment factors representative of relevant concentrations / conditions
Typical Micropollutants			**Typical Legacy Contaminants** 

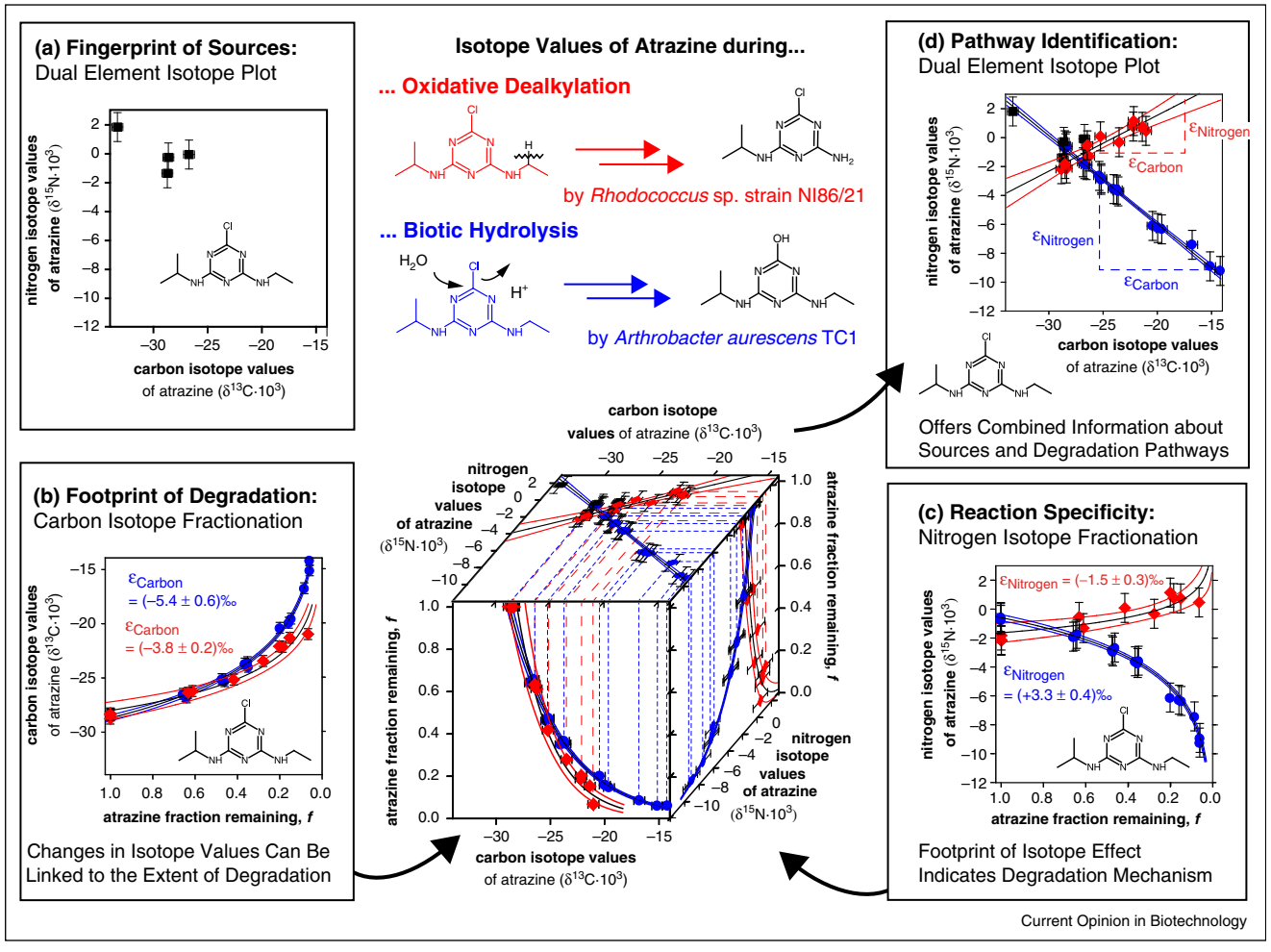
$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{ref}}{(^{13}C/^{12}C)_{ref}} \quad (1)$$

and may serve as fingerprints to evaluate the sources of two otherwise identical substances (Figure 1a). In addition, contaminant molecules with light and heavy isotopes are degraded at slightly different rates, which results in a kinetic isotope effect. Hence, the average isotope value of the residual, non-degraded fraction of the contaminant molecules changes following the Rayleigh equation (Figure 1b) [22,23]:

$$\frac{(^{13}C/^{12}C)}{(^{13}C/^{12}C)_0} = \frac{\delta^{13}C + 1}{\delta^{13}C_0 + 1} = f^{\varepsilon} \quad (2)$$

where f is the residual substrate fraction, $\delta^{13}C$ the corresponding isotope value and $\delta^{13}C_0$ is the source isotope value at the beginning of the degradation ($f = 1$). The isotope enrichment factor ε relates the changes of isotope ratios to the extent of transformation. The ε value can be determined under representative conditions in laboratory experiments using a linear regression according to:

Figure 1



Insights gained from micropollutant CSIA as illustrated by atrazine degradation [24,25•]. **(a)** Fingerprints of sources: isotope values of otherwise identical chemicals differ if they are manufactured from different raw materials or by different synthesis processes. **(b)** Footprint of degradation: isotope values further change due to kinetic isotope effects if contaminants are degraded (here shown for $^{13}C/^{12}C$ in atrazine); the extent of degradation can be quantified according to Eq. (4). **(c)** Degradation mechanisms: the direction and extent of isotope fractionation may indicate the underlying reaction chemistry (here shown for $^{15}N/^{14}N$ in atrazine). In biotic hydrolysis of atrazine (blue graph), for example, an unusual trend to more negative $\delta^{15}N$ values is observed. Protonation of nitrogen atoms during the reaction makes ^{14}N react more rapidly while ^{15}N accumulates in the remaining atrazine (inverse isotope effect) [24]. **(d)** Pathway identification: dual element isotope plots represent $\delta^{15}N$ against $\delta^{13}C$ values in a similar way as in panel (a), only that data from degradation (panel (b) and (c)) is shown. Oxidative dealkylation (red data points) can be distinguished from biotic hydrolysis (blue data points) even without considering concentration data. This circumvents the problem that concentrations in the field decrease due to multiple processes – sorption, dilution and degradation – so that the fraction degraded (f) is typically not accessible. In the dual element isotope plot the slopes of the regression lines are equal to $\varepsilon_{Nitrogen}/\varepsilon_{Carbon}$ and, therefore, reveal the footprint of underlying isotope effects.

$$\ln\left(\frac{(\delta^{13}C+1)}{(\delta^{13}C_0+1)}\right)=\varepsilon\cdot\ln f \quad (3)$$

As a rule of thumb, after 50% of degradation ($f = 0.5$), isotope values (in δ) change by ≈0.7· ε, after 75% of degradation ($f = 0.25$) by ≈1.4· ε, etc. This allows calculating expected changes in isotope values from reported ε values in Table 2 for different degrees of degradation. Negative ε values mean that micropollutant molecules with light isotopes (light isotopologues) are degraded preferentially (normal isotope effect), whereas positive ε values indicate a preferred degradation of heavy isotopologues (inverse isotope effect).

In the environment, micropollutant concentrations mainly decrease by degradation, dilution or sorption, thereby challenging degradation estimates. The inverse form of the Rayleigh equation may provide independent estimates of degradation:

$$B=1-f\left[\frac{(^{13}C/^{12}C)}{(^{13}C/^{12}C)_0}\right]^{1/\varepsilon}=\left[\frac{\delta^{13}C+1}{\delta^{13}C_0+1}\right]^{1/\varepsilon} \quad (4)$$

The extent of (bio)degradation B in the field can thus be retrieved from isotope values alone, without additional information on concentrations or transformation products [18].

Finally, changes in the isotope ratios are often reaction-specific (Figure 1c). Hence, dual element isotope plots may serve to distinguish transformation pathways in environmental compartments and engineered treatment systems if these pathways have previously been linked to specific transformation mechanisms in laboratory experiments (Figure 1d). These dual element isotope slopes are in good approximation given by the corresponding ratios of ε values, (for example, $\varepsilon_{Nitrogen}/\varepsilon_{Carbon}$, Figure 1d) so that they can be estimated for different micropollutants from the data given in Table 2.

Although CSIA is today well established for legacy compounds from contaminated sites [19,20], CSIA of compounds with typical polar micropollutant structures is only emerging (Table 2). Significant changes in isotope ratios of micropollutants could be observed in laboratory experiments (Figure 1 and Table 2). Changes in carbon isotope ratio, however, tend to become smaller with larger molecular size as the non-reactive positions, where isotope values do not change, 'dilute' isotope effects at the reacting bond [26]. As the molecular size of many micropollutants is greater than of typical legacy contaminants (chlorinated C_2-compounds, benzene, toluene), degradation-induced changes in carbon isotope ratios may not be as easily resolved within the variability of commercial sources, unless the source value is well-constrained [27••,28] (Figure 1a). Multielement isotope analysis offers an opportunity to circumvent the problem (Table 2). Compared to legacy contaminants, micropollutants often contain additional elements in their structure. Per element, only a small number of atoms are present (typically 1–3) so that heteroatom isotope effects are less diluted than for carbon. For example, nitrogen isotope fractionation could evidence aerobic diclofenac degradation when carbon fractionation was not significant [29•].

For chiral micropollutants, yet additional insight can be obtained when combining CSIA and enantioselective analysis in so-called enantiospecific stable isotope analysis (ESIA), as first introduced for α-hexachlorocyclohexane (α-HCH) [30]. Enzyme transport and catalysis is frequently enantioselective [31], and enantiomer enrichment may thus indicate biodegradation, in analogy to isotope enrichment [32]. For instance, combining CSIA with enantioselective analysis revealed strong enantiomer and small isotope fractionation during the degradation of phenoxy acid herbicides, which evidenced cell-wall transfer as the rate-determining step of the degradation [33•].

Analytical challenges of micropollutant CSIA as compared with traditional pollutants

The occurrence of very low (sub-μg/L) concentrations of micropollutants and their polarity are the two major analytical challenges which need to be addressed to extend CSIA approaches to micropollutants.

First, enrichment of sufficient analyte (typically a few μg for multiple injections) requires the extraction of large amounts of water (Table 1). While the low volatility of the analytes typically limits their loss during solid phase extraction (SPE), it also hampers matrix cut-off as in purge and trap. During SPE, non-volatile matrix components are thus enriched together with the target compounds, which increases the matrix effect if on-column injection is chosen for optimum sensitivity [36]. CSIA of the atrazine herbicide of the triazine class and its transformation product desethylatrazine in sub-μg/L concentrations could nevertheless be carried out following extract cleanup by preparative HPLC [36]. An extraction and cleanup strategy prior to CSIA also enabled detecting degradation of the pyrethroid insecticide α-cypermethrin in batch soil experiments [75], showing that micropollutants applied or deposited onto soil can be tracked by CSIA. Small isotope fractionation may nevertheless occur in successive sorption–desorption steps in soils and sediments [76,77], and thus should be considered when deriving mechanistic interpretations.

The second challenge lies in the polarity of the micropollutants, requiring the use of a selective sorbent phase

Table 2

Overview of micropollutant CSIA studies. Almost all studies are from the last 10 years. Reported enrichment factors ε (Figure1b,c) indicate the extent of isotope fractionation expected during degradation. After 50% of degradation (f = 0.5), isotope values (in δ) change by $\approx 0.7 \cdot \varepsilon$, after 75% of degradation (f = 0.25) by $\approx 1.4 \cdot \varepsilon$, etc. Dual element isotope slopes (Figure 1d) can be predicted from $\varepsilon_{Nitrogen}/\varepsilon_{Carbon}$.

Substance class	CSIA method development/ validation	Isotope fractionation in laboratory degradation studies	Field applications	Theoretical isotope effect computations
Herbicides				
N-triazine herbicides: atrazine, simazine, ametryn	$^{13}C/^{12}C$, $^{15}N/^{14}N$: GC–IRMS [34] [35] [36]	Hydrolytic biodegradation of atrazine: ε_{Carbon} = (−1.8 to −5.4)‰, $\varepsilon_{Nitrogen}$ = (0.6 to 3.3)‰ [24,37] Hydrolytic enzyme catalysis of ametryn: $\varepsilon_{Carbon} = (-1.9 \pm 0.2)$‰, $\varepsilon_{Nitrogen} = (4.3 \pm 0.4)$‰ [37] Biotic oxidative dealkylation of atrazine and simazine ε_{Carbon} = (−4.0/−4.1)‰, $\varepsilon_{Nitrogen}$ = (−1.4/−1.9)‰ [38] [25•] Phototransformation of atrazine - Direct: $\varepsilon_{Carbon} = (4.6 \pm 0.3)$‰, $\varepsilon_{Nitrogen} = (4.9 \pm 0.2)$‰, $\varepsilon_{Hydrogen} \approx 0$‰ - Indirect: ε_{Carbon}= (−0.5 to −1.7)‰, $\varepsilon_{Nitrogen}$ = (−0.3 to −0.7)‰, $\varepsilon_{Hydrogen}$ = (−25 to −51)‰ [39] Alkaline hydrolysis of atrazine $\varepsilon_{Carbon} = -(5.6 \pm 0.2)$‰, $\varepsilon_{Nitrogen} = (-1.2 \pm 0.2)$‰ [24] $\varepsilon_{Chlorine}$ = (−0.9 to −8.2)‰ [40] [41]	Groundwater samples [36]	Alkaline hydrolysis of atrazine [41] [40] Oxidative dealkylation [25•]
Phenoxyacid herbicides: MCPA, 2,4-D, mecoprop, dichlorprop	ESIA of $^{13}C/^{12}C$: Derivatization + GC–IRMS [42]	Microbial aerobic degradation: ε_{Carbon} = (0 to −2)‰ Enantioselectivity ES = 0.6 to 0.9 (*Sphingobium herbicidovorans* MH) ES = −0.7 to −0.97 (*Delftia acidovorans* MC1) [33•] Enzymatic hydrolysis of mecoprop and dichlorprop methyl esters: ε_{Carbon} = (−2 to −3)‰ [32]	Contaminated site [43]	
Chloroacetanilide herbicides: metolachlor, acetochlor, alachlor	$^{13}C/^{12}C$: GC–IRMS [44•]	Degradation in constructed wetlands: ε_{Carbon} = (−2 to −3)‰ [44•]	Lab-scale constructed wetland [44•]	
Phenyl urea herbicides: isoproturon	$^{13}C/^{12}C$, $^{15}N/^{14}N$: pyrolysis-GC–IRMS [45]	Isotope fractionation in the isocyanate fragment of the isoproturon molecule Abiotic hydrolysis of isoproturon: ε_{Carbon} = (−2 to −5)‰, $\varepsilon_{Nitrogen}$ = (−9 to −11)‰ [46] Bacterial degradation of isoproturon (hydrolytic pathway): $\varepsilon_{Carbon} = (-5.7 \pm 0.2)$‰, $\varepsilon_{Nitrogen} = (-3.3 \pm 0.4)$‰, $\varepsilon_{Hydrogen} \approx 0$‰ Fungal hydroxylation of isoproturon: ε_{Carbon} = (−1.0 to −1.6)‰, $\varepsilon_{Nitrogen} \approx 0$‰, $\varepsilon_{Hydrogen}$ = (−21 to −34)‰ Bacterial degradation of isoproturon (demethylation pathway): $\varepsilon_{Carbon} \approx 0$‰, $\varepsilon_{Nitrogen} \approx 0$‰, $\varepsilon_{Hydrogen} \approx 0$‰ [47]		
Organophosphorous herbicides: glyphosate, AMPA	LC–IRMS $^{13}C/^{12}C$: [48]; GC–IRMS $^{15}N/^{14}N$: [28]; $^{18}O/^{16}O$: [49]	Abiotic oxidation (C, N) [28]; photolysis (O) [49]		

Bentazone	$^{13}C/^{12}C$, $^{15}N/^{14}N$: Derivatization + GC–IRMS [50]		Mesoscale aquifer model [95]	
Chloroanilines (micropollutant surrogate compounds to study direct photolysis)	$^{13}C/^{12}C$, $^{15}N/^{14}N$: GC–IRMS [51]	Direct photolysis at pH 7: 2-Cl-aniline: $\varepsilon_{Carbon} = (+1.5 \pm 0.5)‰$, $\varepsilon_{Nitrogen} = (-2.9 \pm 0.3)‰$ [52•] 3-Cl-aniline: $\varepsilon_{Carbon} = (-0.4 \pm 0.1)‰$, $\varepsilon_{Nitrogen} = (-1.7 \pm 0.2)‰$ [52•] 4-Cl-aniline: $\varepsilon_{Carbon} = (-1.2 \pm 0.2)‰$, $\varepsilon_{Nitrogen} = (-1.2 \pm 0.2)‰$,[53]		
Metabolites/transformation products (parent compound)				
BAM (dichlobenil)	$^{13}C/^{12}C$, $^{15}N/^{14}N$: GC–IRMS [35]	Aerobic biodegradation of BAM: $\varepsilon_{Carbon} = (-7.5$ to $-7.8)‰$, $\varepsilon_{Nitrogen} = (-10.7$ to $-13.5)‰$ [54]	Mesoscale aquifer model [95]	
Desethylatrazine (atrazine)	$^{13}C/^{12}C$, $^{15}N/^{14}N$: GC–IRMS [25•]	Oxidative degradation [25•]		
N-Nitrosodimethyl-amine (NDMA) (different compounds with aliphatic amine functional groups)	$^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$: GC–IRMS [55]			
Substituted N-alkyl anilines (micropollutant surrogate compounds)	$^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$: GC–IRMS [51]	Oxidative transformation (horseradish peroxidase, MnO_2): $\varepsilon_{Carbon} = (-0.1$ to $-2.4)‰$, $\varepsilon_{Nitrogen} = (-7.4$ to $+9.6)‰$, $\varepsilon_{Hydrogen} = (-73$ to $+11)‰$ [56]		
Insecticides				
Hexchlorocyclohexane (HCH) isomers, lindane	$^{13}C/^{12}C$: GC–IRMS [57] (ESIA), [58], [59]	Anaerobic biodegradation (reduction dehalogenation): $\varepsilon_{Carbon} = (-3$ to $-4)‰$ [30] Aerobic biodegradation (isomer-specific and enantiomer-specific fractionation): $\varepsilon_{Carbon} = (-1.5$ to $-1.7)‰$ (ε-HCH); $\varepsilon_{Carbon} = (-2.4$ to $-3.3)‰$ ((+) ε-HCH); $(-0.7$ to $-1.0)‰$ ((−) ε-HCH); [60] Abiotic transformation: $\varepsilon_{Carbon} = (-1.9 \pm 0.2)‰$ (OH radical oxidation), $\varepsilon_{Carbon} = (-2.8 \pm 0.2)‰$ (direct photolysis), $\varepsilon_{Carbon} = (-7.6 \pm 0.4)‰$ (dehydrochlorination), $\varepsilon_{Carbon} = (-4$ to $-5)‰$ (reductive dehalogenation) [61]	Contaminated site [62••]	Dehydrochlorination [63]
Organophosphorous insecticides: dichlorvos, omethoate, dimethoate	$^{13}C/^{12}C$: GC–IRMS: [64]	Abiotic hydrolysis: $\varepsilon_{Carbon} = (-0.2 \pm 0.1)‰$ (dichlorvos), $\varepsilon_{Carbon} = (-1.0 \pm 0.1)‰$ (dimethoat) Photolysis: $\varepsilon_{Carbon} = (-3.7 \pm 1.1)‰$ (dimethoat) [64]		Alkaline hydrolysis of paraoxon [65]
DDT (1,1,1-trichloro-2,2-bis(4-chloro-phenyl) ethane) and metabolites	$^{13}C/^{12}C$: GC–IRMS [58]; $^{37}Cl/^{35}Cl$: GC–MS [66]; Prep. GC + TIMS [67]	Dehydrochloroelimination intramolecular position-specific isotope effect: $\varepsilon_{Chlorine} = 9‰$ [68]	DDT and DDE in seal blubber [69]	

Table 2 (*Continued*)

Substance class	CSIA method development/ validation	Isotope fractionation in laboratory degradation studies	Field applications	Theoretical isotope effect computations
Pharmaceuticals				
Diclofenac	$^{13}C/^{12}C$, $^{15}N/^{14}N$: Derivatization + GC–IRMS [29•]	Aerobic degradation in river sediment: $\varepsilon_{Carbon} \approx 0‰$, $\varepsilon_{Nitrogen} = (-7.1 \pm 0.4)‰$ Reductive transformation with H_2/Pd: $\varepsilon_{Carbon} = (-2.0 \pm 0.1)‰$, $\varepsilon_{Nitrogen} \approx 0‰$ [29•]	Mesoscale aquifer model [95]	
Sulfamethoxazole	$^{13}C/^{12}C$: LC–IRMS [70]	Aerobic biodegradation (*ipso*-hydroxylation): $\varepsilon_{Carbon} = (-0.6 \pm 0.1)‰$ Direct photolysis $\varepsilon_{Carbon} = (-2 \text{ to } -3)‰$ [71]		
Consumer care products				
Benzotriazoles (corrosion inhibitor, dishwashing additive)	$^{13}C/^{12}C$, $^{15}N/^{14}N$: GC–IRMS [27••]	Aerobic biodegradation in activated sludge $\varepsilon_{Carbon} = (-1.9 \pm 0.1)‰$, $\varepsilon_{Nitrogen} = (-1.1 \pm 0.2)‰$ [72]		
Tribromoneopentyl alcohol (TBNPA) (flame retardant)	$^{13}C/^{12}C$: GC–IRMS; $^{81}Br/^{79}Br$: GC–MC–ICPMS [73]	Alkaline hydrolysis: $\varepsilon_{Carbon} = (-10.4 \pm 1.6)‰$, $\varepsilon_{Bromine} = (-0.4 \pm 0.1)‰$ Oxidative degradation with H_2O_2/CuO: $\varepsilon_{Carbon} = (-10.4 \pm 1.6)‰$, $\varepsilon_{Bromine} \approx 0‰$ Reductive dehalogenation by Fe(O): $\varepsilon_{Carbon} = (-7.6 \pm 0.7)‰$, $\varepsilon_{Bromine} = (-1.9 \pm 0.2)‰$		
Phthalates (plasticiser)	$^{13}C/^{12}C$: GC–IRMS [74]	Aerobic biodegradation: very small carbon isotope fractionation [74]		

for SPE and/or a derivatization step prior to GC separation (see Table 2). As heteroatoms, such as N, can increase sorption to metal surfaces, the use of glass connectors and online conversion conditions (that is, choice of metals and operating temperature) in the combustion oven of GC–IRMS were found to be critical for precise and accurate CSIA [27••,35].

The challenges posed by low concentrations and compound polarity also mirror the current limits of environmental ESIA applications for chiral micropollutants, namely, first, the large amount of target analyte required (that is, 10–100 ng per injection), second, strong matrix effects due to the low operating temperature of chiral gas chromatography (often <250 °C), and third, poor chromatographic resolution with insufficient baseline separation ($R < 3.0$) [78].

Prospects of micropollutant CSIA in field studies

Although the above-mentioned analytical challenges currently limit field studies of micropollutant CSIA, recent advances summarized in Table 2 and Figure 2 show that applications in the environment are now within reach, and can be foreseen in different situations of increasing complexity. First, in analogy to steady-state assessments of petroleum or chlorinated hydrocarbons, evidence of degradation is warranted for cases of *continuous pesticide release* from point sources. [79] Second, although more challenging, evidence of degradation is also sought when micropollutant pulses are tracked in *event-based studies* relative to a conservative tracer to delineate 'hot spots' and 'hot moments' (for example, [80]). Third, comprehensive assessments of degradation on the catchment scale, which attempt to integrate over a season *multiple micropollutant sources and events*, are typically not able to detect where, when and how degradation occurs [81] calling for innovative approaches. Forth and finally, investigations of pesticide degradation in groundwater over years are presently elusive, because current methodological approaches are not able to unequivocally detect degradation in *surveys over longer time scales*. In the following section, the potential of CSIA is discussed to deliver insight in these different situations of increasing complexity.

(a) For *steady-state conditions*, CSIA was able to differentiate hexachlorocyclohexane (HCH) sources and identify HCH biodegradation [62••]. To this end, enantiomer-specific enrichment factors [60], and validated instrumental methods [57,59] are available. For instance, the identification of phenoxy acid herbicide degradation (dichlorprop and its degradate 4-CPP) with spatially varying enantioselectivity underscored the advantages of combining enantiomer ratios, CSIA and ESIA at a geologically complex landfill [43].

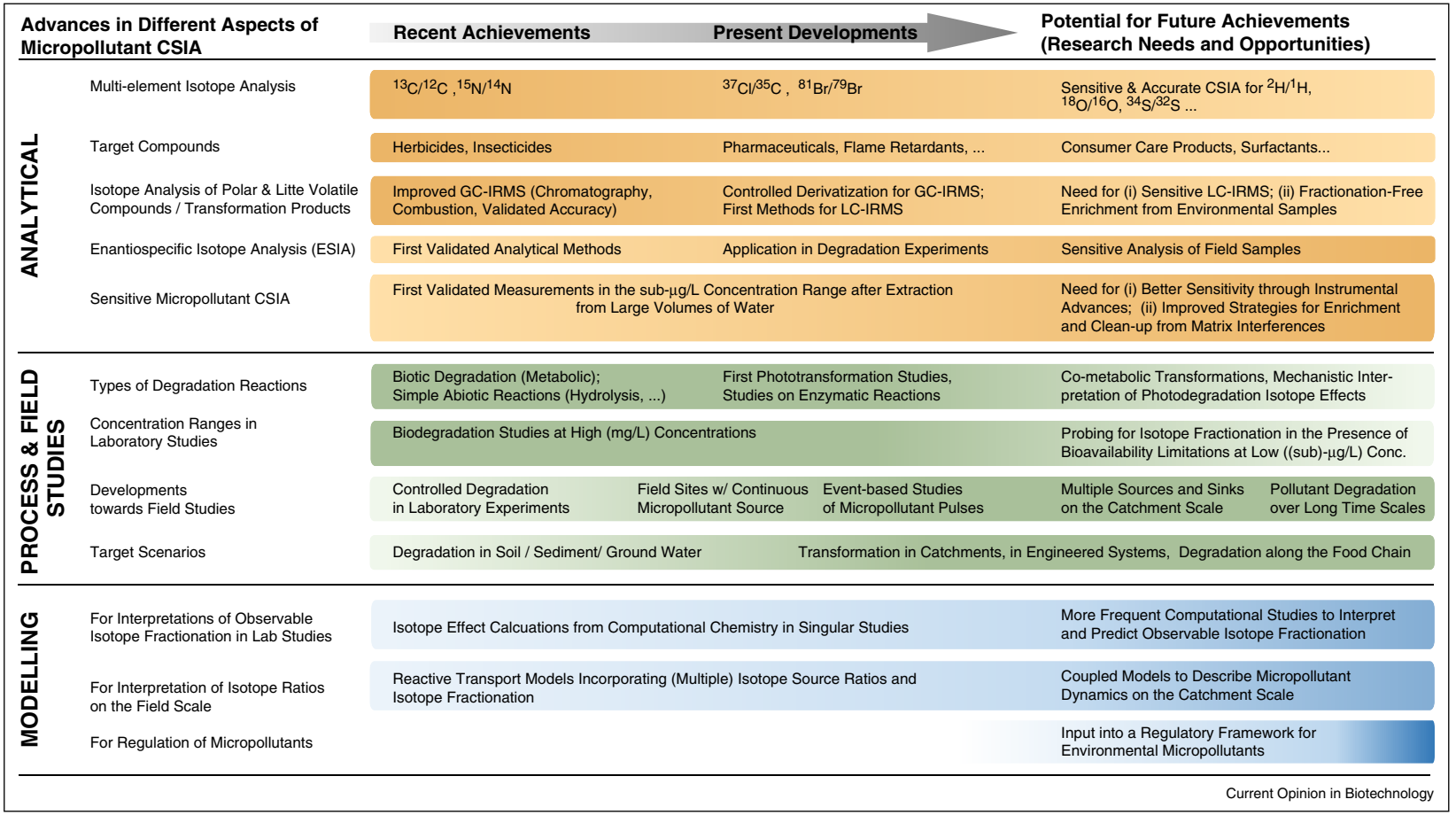
Complementarily to field studies, smaller-scale and controlled environmental systems can be used to develop, test and validate CSIA approaches under steady-state or event-based conditions. For instance, CSIA enabled to identify the degradation of chloroacetanilide herbicides in lab-scale wetlands [44•]. Similarly, aerobic degradation of the corrosion inhibitor benzotriazoles was studied in batch experiments with activated sludge [72]. Such laboratory experiments in complex but controlled systems underline the interest in micropollutant CSIA to evaluate the performance and reliability of polishing wetlands or wastewater treatment plants.

(b) In *event-based studies*, the isotope data of micropollutants in surface water may help to interpret the heterogeneity of reactive compartments and transformation pathways. Although such field studies have not been reported yet, a coupled subsurface–surface reactive transport model (HydroGeoSphere) incorporating CSIA data was applied at the hillslope scale [82•]. The model showed that CSIA can support the identification of rainfall–runoff events transporting pesticides to the stream or the transport routes and, more largely, the evaluation of diffuse river pollution. Outputs of models including CSIA can be confronted in the future with data acquired at different time and spatial resolution on the catchment scale or in engineered treatment systems, and enhance the interpretation of sources and dissipation of pulses of diffuse contaminants. The ability of CSIA to track degradation of such a transient micropollutant pulse was recently demonstrated in a mesoscale aquifer [95].

(c) In the future, micropollutant CSIA may also serve comprehensive studies on a *larger spatial scale with multiple sources and events* over a season. Integrative strategies relying on isotope data have been conducted for more than three decades in the case of nitrate, and significantly contributed to the development of water management policies [83,84,85]. The important lessons learned are that firstly, a detailed sampling strategy needs to be developed based on possible sources, hydrology and land use, and secondly, nitrate isotope data should be interpreted together with physicochemical and hydrological tracer data to evaluate the pollution sources and the processes that nitrate has undergone during its retention and transport in a catchment.

(d) Micropollutant CSIA also offers a unique opportunity to trace degradation of persistent organic pollutants *over longer time scales* [69] as information from CSIA is independent from mass balances and time scales. For example, CSIA may help to understand why herbicides such as atrazine remain frequently detected in groundwater even decades after their ban in Europe.

Figure 2



Current development and future prospects to evaluate micropollutant fate in the environment using CSIA.

Conclusions and current research needs

Future research needs concern analytical and conceptual issues, at both the molecular and the environmental scale (Figure 2).

From an analytical viewpoint, the extension of CSIA methods (Table 2) to new compounds (flame retardants, surfactants, stabilizers, etc.), additional elements [86] and chiral micropollutants is expected to boost the evaluation of micropollutant degradation (Figure 1). In the future, even modest instrumental advances will enhance environmental monitoring of micropollutants using CSIA. For instance, even small improvements of the sensitivity and the peak separation of GC–IRMS (for example, by one order of magnitude) and in the efficiency of enrichment, cleanup and injection methods will help reducing the collected water volumes to manageable sizes (for example, 5 L instead of 50 L) and lower matrix interferences. In addition, as transformation pathways of most micropollutants are still unknown, complementary non-target analysis of transformation products [72] is expected to considerably advance the interpretation of isotope fractionation.

To interpret micropollutant CSIA field data and respond to the research gaps addressed here, there is also a need to characterize fundamental processes at the molecular scale. Additional isotope fractionation data from laboratory experiments on enzymatic transformation [25•,87] or photodegradation [52•,53] are necessary to tackle issues such as trophic transfers of persistent micropollutants or degradation in engineered systems (see Table 2). In addition, low micropollutant concentrations call into question the representativeness of ε-values retrieved in laboratory experiments with higher concentrations (Eq. (3), Table 2). Depending on whether substrate uptake into the microbial cell, or slow enzyme kinetics is rate-determining, the extent of stable isotope fractionation may also be smaller than expected [88•]. Finally, micropollutants at low concentrations may serve as non-growth substrates and their co-metabolic transformation may be coupled to the metabolism of other substrates of the DOC [89]. Therefore, better knowledge of the kinetics of co-metabolic micropollutant transformation is required to reliably interpret isotope fractionation in the field.

How micropollutant degradation influences isotope fractionation under field-relevant conditions may be addressed in numerical [90] and benchmark experiments based on chemostat or homogeneous porous systems. Such experiments may help to determine factors governing isotope fractionation of firstly, the micropollutant concentration (or DOC/micropollutant ratio), secondly, the mean residence time of the micropollutant at the linear pore velocity scale (see [91•]), and finally, its diffusion time at the pore scale.

Modelling approaches may also catalyze CSIA applications and upscale in increasingly complex systems, including multiple sources, transformation pathways or reactive zones and periods, by assisting in the experimental design and the data interpretation. On the molecular level, computation of isotope effects may push forward the theoretical basis for the interpretation of underlying mechanisms and isotope fractionation (see Table 2). Complementarily, mathematical models including CSIA concepts and data [92] may help delineating multiple contaminant sources and sinks [93] in aquifers or engineered systems [94], or quantify the evolution of micropollutants on the catchment scale [16] to eventually feed into a regulatory management framework.

In summary, recent advances in micropollutant CSIA open the door to the development of field-based investigations and applications. The speed and impact of this development will mostly depend on the ability to address the research needs summarized in Figure 2. Micropollutant CSIA, when combined with soil and hydrological characterization, identification of transformation products and modelling, may significantly contribute in the future to identifying degradation hot spots and hot moments on the catchment scale or micropollutant degradation over long time scales. By providing missing information on micropollutant degradation and cycling, such information is expected to feed into legislation and regulation of micropolluant use and management.

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